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ARTICLE XV.

On the Organization of the Genus Gregarina of Dufour. By Joseph Leidy, M.D. Read January 3, 1851.

In 1826, Dufour described in the *Annales des Sciences Naturelles*, an entozoic parasite found within the intestinal canal of various coleopterous insects. He gave a general account of its structure, and notices its apparent analogy to the genus of intestinal worms, *Caryophylleus*, of Rudolphi, and also remarks, p. 45, that Ramdohr has represented the same animal under the name of "Petit sac de l'Epiploon," found in the *Dermestes lardarius*.

In 1828, Dufour, in the same work, characterized the parasite as a new and distinct genus of entozoa, under the name of *Gregarina*, "que exprime l'habitude qu'ont ces vers intestinaux de vivre en troupes." Those infesting Coleoptera, he designated under the general specific name of *Gregarina conica*, those found in the *Forficula*, he called *Gregarina ovata*. The author gives three figures of the latter, one of which represents an attached pair.

In 1838, Hammerschmidt indicated, in the *Isis von Oken*, a number of species of *Gregarina*, which, with very little reason, he subdivided into five genera.

Siebold, in 1829, Kölliker, in 1845, Henle, in the same year, Frantzius, in 1846, and Stein, in 1848, wrote upon the character of the *Gregarinæ*; but to their writings I have not been able to have access.

In 1848, Kölliker wrote a second time on the nature of *Gregarina*, in Siebold and Kölliker's *Zeitschrift für Wissenschaftliche Zoologie*, vol. i., p. 1, in which he contends that this singular helminth is a single, simple organic cell,—an opinion he held in his former memoir, and which, according to this author, was questioned by Henle and Frantzius.

In the second part of the latter memoir of Kölliker, p. 18, on the general views of the nature of *Gregarinæ*, he asks "Sind die *Gregarinen* Thiere?" (are the *Gregarinæ* animals?) a question which arose from an opinion expressed by Henle, from their relationship to the receptacles of the *navicellæ*, which latter are usually considered as vegetable in their nature. In answer to the question, the author says, the contractility of the membrane and its solubility in acetic acid, speak pretty safely for the animal nature of the *Gregarinæ*, as no contractile cell membrane soluble in acetic acid is yet known among plants.*

* Loc. cit. p. 18. "Die Contractilität der Membran derselben und zweitens deren Auflöslichkeit in Essigsäure ziemlich sicher für die thierische Natur dieser Wesen sprechen, da man bis jetzt bei Pflanzen durchaus keine contractile und in Essigsäure lösliche Zellmembranen kennt."

Besides, observing that the peculiarity of the movements is more like that of animals than of plants, he finally states, that the habitation of the *Gregarinæ* is such as is frequent among lower animals, and but seldom among plants.

Without at all entering into the views of Henle, in considering the *Gregarinæ* of a vegetable nature, I must state in opposition to Kölliker, that contractility of cell membrane does exist in the vegetable kingdom, if the *Achlya proliferæ* be regarded as a plant, for its spores after escaping from the sporangium do not only move by means of vibrillæ, but there is also a very evident degree of contractile movement existing in their membrane. In relation to the habitation of plants being but rare in animals, my observations lead me to consider the occurrence of plants growing within healthy living animals as by no means an unfrequent one.*

In answer to a second question proposed by the author, "Sind die *Gregarinen* einzellige Thiere?" (Are the *Gregarinæ* single-celled animals?), he regards the integument of these animals as cell membrane, the fluid and granular matter within as cell contents, and the clear corpuscle, among the latter, as a cell nucleus containing a nucleolus. This is the most important opinion as to the character of the parasite, and is the one most objected to by Henle and Frantzius, who contend that the interior corpuscle is not of the nature of a cell nucleus.

Kölliker appears to have been mostly influenced in forming an idea as to the nature of the *Gregarina*, from its close relationship to the *Monocystis*. The division of the contents in the two parts of the body he regards as not being a particular membranous partition, but consisting of the same clear, tough fluid, which binds the granules of the contents together.†

The probosciform appendage possessed by many species, the author considers as no objection to the simple cell nature of the animal, and as instances of similar cells, refers to the pollen granule, among plants, which often has prolonged growths, (*Auswüchse*), and the capillaries of the larva of frogs in the course of development, among animals, which then have prolonged solid growths of the cell membrane.

Frantzius, in some concluding remarks upon *Gregarinæ*, in the *Archiv für Naturgeschichte*, for 1848, p. 190, remarks, that the partition separating the contents of the animal into two parts, he does not consider, with Kölliker, as a thickened layer of the fluid of the body, but, with Stein, thinks it is a partition from the general integuments of the body, which, he continues, is perhaps foreign to the conception of the true cell.‡

Kölliker is so satisfied of the simple cell character of the parasite that in the second vol. of the *Zeitschrift für Wissenschaftliche Zoologie*, p. 114, he observes, hardly any body will doubt the *Gregarinæ*, with their structureless membrane, simple contents, and nucleus, are in the highest degree like a common cell.

My observations on the genus *Gregarina* of Dufour, lead me to consider it as occupying a much higher position among helminths than has been generally attributed to it, and with Frantzius, Stein, and Henle, as not being a simple organic cell.

* See Proc. Acad. Nat. Sci. vols. 4, 5.

† Loc. cit. p. 22.

‡ Die in den Gregarinen vorkommende Scheidewand, die ich nicht wie Kölliker nur für ein verdickte Schichte des flüssigen Körper-inhalts, sondern mit Stein ebenso wie die übrige Körperhülle für eine Membran halte, ist einmal etwa dem Begriff der wahren Zelle Fremdartiges.

The *Gregarina* is a very simple form of entozoon infesting insecta, crustacea, myriapoda, and annelida. In insecta and myriapoda I have usually found them within the proventriculus, but occasionally within the cavity of the abdomen applied to the exterior parietes of the organ just mentioned. They are usually more or less wrinkled or flaccid, except in the full-grown condition, when they are more or less distended with the granular contents.

When the gastric fluid in which the parasite is found is diluted with water, it becomes distended by rapid endosmosis, and soon undergoes destruction. The fluid which I used in examining them, and which I generally use in investigating the delicate tissues of insects, is the blood of the latter. The *Gregarinæ* are not always loose and floating, but are frequently found attached by the posterior end to the delicate transparent epithelial layer, which is so often seen detached in the form of a cylinder from the interior parietes of the proventriculus of insects, and when the animals are separated they may often be observed with a shred of membrane attached, as represented in fig. 8, pl. 10.

A *Gregarina* consists of two portions or divisions of the body, which, for convenience, I will call the cephalic and posterior sac, the former of which is considerably smaller in size than the latter, and is placed anteriorly upon it, or partly in a depression in it, and both are intimately connected together by the tegumentary tunic.

Each sac contains within the interior a mass of granular matter, which, according to the quantity, will appear whitish and translucent, or white and more or less opaque, although the constituent granules of the masses are transparent, resembling oil granules, and measure from a mere point to about the 1.7500th of an inch. The granules are smaller and fainter in the young animals, and in the oldest individuals they exist to such an extent as to give them a milk-white opaque appearance. Frequently there are some granules of a larger size than ordinary mixed with the others, but still preserving the same structure.

Tincture of iodine renders the masses of granular matter brown; ether causes many of the granules to run together and form large globules.

The granules of the masses are held together by a clear, colourless, viscid, albuminoid fluid, which is coloured brown by iodine, and faintly yellow by nitric acid and ammonia.

The parietal tunic of the sacs is colourless, transparent, and structureless, or amorphous in structure. A partition of the same character passes between the two sacs, separating the granular masses from each other.

This tunic is softened or even dissolves in acetic acid; iodine turns it brown; nitric acid and ammonia faintly yellow.

It completely closes the posterior sac from all communication with the exterior and also from the interior of the cephalic sac.

It forms the cephalic sac, which appears also to be closed from the exterior. At the anterior part of this sac it is frequently thickened into a papillary eminence, and sometimes is prolonged into a probosciform appendage. I could never ascertain beyond all doubt whether there was not a communication from the interior with the exterior, through this anterior thickening of the tegument. When the animal is submitted to pressure, both sacs may be burst, rarely one into the other, or through the papillary thickening of the

tunic of the cephalic sac, but most commonly at some part of the sides, allowing the contents to escape.

Within the parietal tunic of the posterior sac is a second membrane, which is transparent, colourless, and marked by a most beautiful set of exceedingly regular, parallel, longitudinal lines, which in *Gregarina Juli marginati* measure the 1.9375th of an inch apart; in *G. Blattæ orientalis* the 1.10.000th of an inch; and in *G. Passali cornuti* the 1.15.000th of an inch. This tunic has entirely escaped the notice of all previous observers, and I can account for the circumstance in no other way, than by supposing it has arisen from the inferiority of the microscope made by European continental artists. The lines or markings are easily observed, without any other than the ordinary arrangement for light, by the $\frac{1}{4}$ of an inch, but better the $\frac{1}{12}$ th of an inch focal power of the instrument of Messrs. Powell & Lealand.

Of course, if the existence of this second tunic be confirmed, and I have seen it too frequently and plainly to think I have been deceived, the idea of the *Gregarina* being a simple organic cell, is at once exploded.

This tunic I did not detect in the cephalic sac, it appears to go only to the partition dividing the latter from the posterior sac. I have considered it as a muscular or sarcodic structure, because the posterior sac alone is endowed with movement, and as the parietal tunic is the same in both sacs, it is reasonable to conclude the contractile power resides in this second tunic.

Situated in the granular mass of the posterior sac, is to be found one, sometimes two, globular, transparent, colourless, homogeneous, elastic corpuscles, which measure from the 1.4000th to the 1.275th of an inch in diameter. The elasticity of these bodies is so marked, that I have occasionally observed them pressed into a cylindrical form, or constricted into an hour-glass shape, by the contraction of the posterior cell, and upon removal of pressure they would instantly resume their usual appearance.

It is sometimes faintly granular, at other times, in its homogeneous mass may be seen a few scattered, minute, transparent granules. Frequently it contains a nuclear body which is globular, transparent, and homogeneous, with or without one or two nucleoli. Sometimes the nucleus is coarsely granular. Occasionally the corpuscle contains several nuclei.

Movements. Upon the endosmosis of water or saliva into the *Gregarina*, a quite active degree of molecular movement is observable among the granules of the contents.

The contractile movements of the animals, as before observed, take place only in the posterior sac. These appear to be of a muscular character, to such a degree that I was led to the detection of the muscular tunic in seeking for their source. They are slow, and resemble very much the movements produced by the contractile fibres of the dartos membrane of the scrotum.

The posterior sac contracts in any part of its extent without necessarily involving any other part, or it may contract simultaneously throughout. The movements consist of a slow bending of any part of the posterior sac, or constriction of its parietes, or involution of any part, or contraction of the cephalic sac with involution, or general contraction removing any involution, with projection of the cephalic cell if it had been previously retracted.

The older *Gregarinæ*, when distended with granular contents, frequently are quite motionless.

Among the *Gregarinæ* of twenty or thirty different insects and myriapoda, I have preserved notes only of seven, which I append to this communication.

CHARACTER OF THE GENUS.

GREGARINA, Dufour.

Dufour, An. d. Sc. Nat. T. 8, 1826.

Gregarina.—*Dufour*, An. d. Sc. Nat., 1828; *Hammerschmidt*, Isis von Oken, 1838; *Siebold*, Beitr. z., Naturg. d. wirbellosen Thiere, 1839; *Kölliker*, Zeits. f. wiss. Botanik, 1845; Zeits. f. wiss. Zoologie, 1848; *Henle*, Müller's Archiv, 1845; *Frantzius*, Observat. quædam de Gregarinis, 1846; Archiv f. Naturges. 1848; *Stein*, Müller's Archiv, 1848.

Clepsedrina, *Rhizinia*, *Pyxinia*, *Bullulina*, *Hammerschmidt*, Isis von Oken, 1838.

Sporadina, *Stylorhynchus*, *Actinocephalus*, *Frantzius*, Archiv f. Naturgeschichte, 1848.

Body consisting of two sacs connected together, composed of amorphous membrane, and filled with granular contents. Anterior or cephalic sac much the smaller. Posterior sac lined with a second tunic, marked with exceedingly regular, parallel, longitudinal lines. Granular mass of the posterior sac containing one or two globular, transparent corpuscles.

1. GREGARINA JULI MARGINATI.

Gregarina larvata, *Leidy*, Proc. Acad. Nat. Sci., vol. iv., p. 232.

Opaque, white, cylindrical or fusiform, narrowed posteriorly, frequently considerably dilated at the anterior third.

Anterior or cephalic sac small, oblate spheroidal, received about one-half into a depression of the inferior cell, surmounted by a papillary elevation or thickening of the parietal integument, which often appears as if there were outlines of a canal or communication with the interior in it; interior filled with a finely granular mass, resembling an aggregation of oil globules; granules measuring from 1.15,000th to 1.7,500th of an inch.

Posterior sac, elongated, cylindrical, or fusiform, obtuse posteriorly; interiorly filled with a granular matter like that of the cephalic sac, rendering the larger or older individuals opaque from its quantity, and one or two large, globular, transparent, homogeneous corpuscles, containing a fine, granular matter, and a round, granular, or transparent nuclear body, in older individuals with one or two nucleoli; interior of the parietal integument invested with delicate and exceedingly regular parallel lines, about the 1.9375th of an inch apart.

Whole length of animal from the 1.200th to the 1.30th of an inch.

Cephalic sac of largest individual, 1.800th in. long; 1.615th in. broad; of smallest 1.1232d in. long.

Breadth of posterior sac, from the 1.830th in. to the 1.111th in.

Corpuscle of the posterior sac, in the larger individuals, the 1.375th in. in diameter; its nucleus the 1.967th in.; the nucleolus the 1.1666th in.

Habitation.—Found in considerable numbers in the proventriculus of *Julus marginatus*, in about two-thirds of the animals examined.

Movements.—This is the most active species which I have ever observed. Individuals are found, generally those of largest size, which are frequently motionless. The movements consist of a contraction, more or less extensive, of the posterior sac. This occurs sometimes to such an extent, that the body will be so narrowed that the parietes come in contact, and the granular contents are divided into two portions, one anterior and the other posterior to the constriction. It will also bend into a sigmoid posture, or roll itself into a helix. At times the cephalic sac is totally drawn within the posterior, and again projected.

2. GREGARINA JULI PUSILLI.

White, translucent, oval.

Cephalic sac hexahedral, with the sides rounded, or forming a double cone, base to base, with the upper apex subacute, or truncated in younger individuals.

Posterior sac robust, oval; granular contents, fine, translucent; interior corpuscle, globular, transparent; nucleus transparent, without nucleolus.

Whole length from the 1.1500th in. to the 1.275th in.

Breadth of largest the 1.500th in.

Diameter of head of largest, 1.1500th in.

Habitation.—Intestine of *Julus pusillus*.

Movements.—Its movements are not frequent.

3. GREGARINA POLYDESMI VIRGINIENSIS.

White, translucent, clavate, spatulate, or oval.

Cephalic sac campanulate, globular, or prolate, or oblate spheroid, surmounted by a papillary thickening of the integument; interior granular mass very fine and translucent.

Posterior sac globular, oval, clavate, spatulate, fusiform; or urceolate; posteriorly obtuse; parietal integument wrinkled or distended; granular contents very fine, faint, translucent; corpuscles, one or two, globular, transparent, very faintly granular.

Whole length from the 1.1000th in. to the 1.28th in.

Breadth from the 1.1000th to the 1.430th in.

Cephalic sac in largest 1.1400th in. long.

Corpuscle 1.3000th to the 1.1000th in. in diameter.

Habitation.—Intestine of *Polydesmus virginienensis*.

Movements.—Moderate and slow.

4. GREGARINA PASSALI CORNUTI.

White, opaque, in pairs, wrinkled.

Cephalic sac hemispherical, without papillary thickening of the integument, interior granular mass opaque.

Posterior sac flaccid, when distended subglobular; granular contents opaque white, completely obscuring the interior corpuscle.

Average length, in pair, after distension, 1.66th in.; breadth, the 1.133d in.

Cephalic sac 1.260th in. at base; projects from body cell 1.533d in.

Muscular lines of the posterior sac 1.25000th in. apart.

Habitation.—Proventriculus of *Passalus cornutus*.

Remarks.—This species is usually found in pairs, the cephalic sac of one applied to the posterior part of the posterior sac of another; a disposition from which Hammerschmidt referred it to a new genus under the name of *Clepsidrina*. A very trifling degree of pressure is sufficient to separate them from one another.

Movements.—I never observed any other movement in this *Gregarina* than a slight contractile one, commencing at the anterior part of the posterior sac and proceeding backward.

5. GREGARINA ACHETÆ ABBREVIATÆ.

White, opaque or translucent, with or without a probosciform appendage.

Cephalic sac orbicular, or oval, without any papillary thickening of the integument, or occasionally with an oval probosciform prolongation; interior granular mass opaque or translucent.

Posterior sac obconic; granular contents opaque; interior corpuscle transparent.

Whole length from the 1.300th to the 1.70th in.

Breadth from 1.600th to the 1.130th in.

Cephalic sac from 1.1140th in. to the 1.250th in.

Habitation.—Proventriculus of *Acheta abbreviata*. The same species I have observed in two instances on the exterior of the ventriculus, free within the abdominal cavity.

Movements.—Very slow.

6. GREGARINA LOCUSTÆ CAROLINÆ.

Opaque, white, with or without a proboscidiiform appendage.

Cephalic sac hemispherical, without papillary thickening of the integument, or occasionally with a pyramidal enlargement or extension anteriorly, terminating in a round or oval proboscidiiform prolongation, fringed with delicate, membranous digitations at the free extremity, or with an apparent depression of the parietal integument, and a slight conical protrusion of the interior granular mass, which latter is opaque. Partition between the contents of the cephalic and posterior sac very thin.

Posterior sac oblong oval, or spatulate; posteriorly obtuse; granular contents, opaque; interior corpuscle transparent, with several nuclear bodies.

Whole length from the 1.100th to the 1.60th in.

Breadth from the 1.200th to the 1.120th in.

Cephalic sac from the 1.400th to 1.320th in. long; from the 1.270th to the 1.178th in. broad.

Corpuscle, in largest, 1.2800th in. diameter; nuclei 1.3500th in.; nucleoli 1.7000th in.

Habitation.—Intestine of *Locusta Carolina*.

Movements.—None observed.

7. GREGARINA BLATTÆ ORIENTALIS.

Robust, milk-white, opaque.

Cephalic sac hemispherical, with a slight papillary thickening of the integument; contents opaque white. Partition between the cephalic and posterior sac thin.

Posterior sac broad ovate, or panduriform, subacute posteriorly; contents opaque, obscuring the interior corpuscle.

Whole length average 1.50th in.; breadth 1.125th in.

Cephalic sac 1.260th in. long; 1.178th in. broad at the base.

Corpuscle 1.275th in diameter.

Muscular striæ 1.10.000th in. apart.

Habitation.—Within the intestine, and without closely applied to its parietes, in *Blatta orientalis*.

Movements.—The movements of this species are moderately active.

EXPLANATION OF THE FIGURES. (PLATES 11, 12.)

The figures are all magnified and viewed by transmitted light.

Figs. 1 to 20.—*Gregarina Juli marginati*.

Fig. 1.—An ordinary form in a state of rest; length 1.25th in.; breadth 1.160th in.

Figs. 2 to 8.—Exhibit various movements of the animal. To the posterior part of figure 8 is observable an attached shred of epithelium.

Figs. 9 to 12.—Represent younger animals in movement; fig. 9 1.80th of an inch long; fig. 10 1.107th in.; fig. 11 1.160th in.; fig. 12 1.57th in.

Fig. 13.—An individual, highly magnified, exhibiting the appearance of the contained granular masses, and also presenting to view two globular, nucleated, corpuscles.

Fig. 14.—The posterior extremity of the same individual more highly magnified. The larger granules at the bottom exhibited very lively molecular movements.

Fig. 15.—A similar individual with the two sacs burst from pressure, and the contained matters escaping. The longitudinal markings of the inner tunic of the posterior sac are represented.

Fig. 16.—Posterior extremity of the same individual, with the contents removed, exhibiting the arrangement of the longitudinal lines.

Fig. 17.—Anterior extremity highly magnified. An apparent canal or opening is represented as existing in the papillary thickening of the integument.

Fig. 18.—Exhibits the relation of the longitudinally lined tunic of the posterior sac to the cephalic sac.

Figs. 19, 20.—The two globular nucleated corpuscles of the posterior sac.

Fig. 21.—*Gregarina Juli pusilli*. Length 1.250th in.

Fig. 22.—A younger individual. Length 1.1500th in.

Figs. 23 to 29.—Various forms and ages of *Gregarina Polydesmi virginiensis*.

Fig. 30.—An attached pair of *Gregarina Passali cornuti*.

Fig. 31.—A single individual of *Gregarina Passali cornuti*, in outline, representing the longitudinal lines of the posterior sac.

Fig. 32.—*Gregarina Achetæ abbreviatæ*.

Fig. 33.—A young individual of *Gregarina Achetæ abbreviatæ*.

Fig. 34.—A young individual of *Gregarina Achetæ abbreviatæ*, with a probosciform appendage.

Figs. 35, 36.—*Gregarina Locustæ Carolinæ*.

Fig. 37.—Another individual of *Gregarina Locustæ Carolinæ*, with a remarkable probosciform appendage.

Fig. 38.—Corpuscle of the posterior sac of the same individual, with numerous nuclei.

Figs. 39 to 41.—Three forms of *Gregarina Blattæ orientalis*.